A METHOD FOR OBTAINING SPORES OF THE FUNGUS BEAUVERIA BASSIANA (BALS.) VUILL. IN QUANTITY

E. E. McCoy and C. W. Carver
New Jersey Department of Agriculture, Trenton, N. J.

From time to time the possibility of applying entomogenous fungi as control measures for insect pests arouses interest among entomologists. Unfortunately most attempts to use fungi in practical control work have not met with marked success and yet, under natural conditions, wide-spread epidemics of some of these fungous diseases occur and cause drastic reductions in their insect hosts. Possible reasons for these failures may be: too light spore dosages employed; too small areas involved; repeated applications not made.

Many workers would undoubtedly use heavier or repeated spore distributions if it were possible to obtain a larger quantity of spores without a disproportionately large amount of culture work. The authors feel that the procedures here described will at least alleviate this latter difficulty. While the methods described apply specifically to the culture of *Beauveria bassiana*, it appears probable that they are subject to adaptation to a number of fungi. They have already been applied by us to an *Aspergillus* sp. recently isolated from diseased sawfly adults.

A number of the entomogenous fungi offer no particular difficulty from the standpoint of artificial culture, and have been grown on various media. Many of these media are essentially some type of nutrient agar. *Beauveria* grows well on a variety of agar media, and has also been grown on corn meal mush (1) and soy bean mash (2), for example. The yield of spores from such cultures, large though it appears in the laboratory, seems small when one considers spreading the material over an area of several acres. Other workers have recognized this difficulty.

The authors became interested in the culture of *Beauveria* as a result of recent experiments which indicate that the fungus readily parasitizes the Japanese beetle adult (3). The spores

used in the initial phases of this work were grown in Petri dishes, using potato agar as the culture medium. It was soon evident that a better culture method would be necessary if any extensive work was to be done. *Beauveria* is not fastidious, and was found to grow on various agar media, corn meal mush, soy bean mash, sweet potato, bread and a number of other materials. However, the best growth by far occurred on autoclaved, moistened, bran. Bran does not appear to have been widely used in mycological work, but possesses certain physical properties, as well as nutrients, which adapt it excellently for the problem. Foremost among these is that fungi fruit, or bear spores, on the surface of the medium, and the bran particles present an enormous surface. Spore production, in bran cultures, therefore becomes practically a three dimensional effect. It is evident that a wide range of nutrient solutions, or solid materials, can be mixed with the bran if necessary to promote growth of the more fastidious fungi.

The wheat bran employed is the ordinary kind used for feeding livestock and is obtainable at any feed store. The adherent and intermixed starch, etc., supply the nutritional requirement of the *Beauveria*. The *Beauveria* used in this work was obtained originally through Dr. M. Timonin, who described the strain as a parasite of the Colorado potato beetle (4). This strain, however, was later used to infect a series of Japanese beetle adults and re-isolated from the latter insect by us. The final fungus strain appears to have growth habits which differ somewhat from the original material, possibly as a result of unintentional selection, or selective adaptation. At any rate it seems to sporulate more rapidly and compactly than does the original material.

The bran is moistened with tap water, using 1 part by weight of bran to 1.5 parts by weight of water; as 100 gms. bran plus 150 ml. of water. The mass is thoroughly mixed. The moistened bran is placed in flasks, the latter plugged, and autoclaved. Experience has shown that a 30-minute period at 15 lbs. steam pressure is sufficient to sterilize the medium in a 2-liter Erlenmeyer flask. It is best not to have the flasks more than half filled with medium.

After the autoclaved medium has cooled it is inoculated with the fungus. A small amount of a matured bran culture makes

an ideal inoculum. The inoculum is thoroughly distributed by shaking the plugged flask. The culture may be incubated in the flask, in which case it is desirable to shake the flask occasionally to break the extremely dense mycelial development, and maintain the contents in a more or less granular condition. More rapid growth occurs if the inoculated bran medium is spread in sterile Petri dishes, maintaining the mass as loose as possible. Culture dishes 200 mm. in diameter by 20 mm. high will contain roughly the equivalent of 60 gms. of bran (150 gms. medium) each. Such a culture yields approximately 3 gms. of spores. The yield is therefore about 22 gms. of spores per pound of bran. If the spores were applied at the rate of 20 gms. per acre, as has been done in some experimental work with the corn borer, the culture material would cost $0.02 per acre, and 7 of the 8″ culture dishes would be required.

The rate of development of the *Beauveria* depends upon temperature. The optimum incubation temperature is 80–85° F. At 72° F. these cultures are customarily incubated for from 10 to 15 days. After incubation the cultures are removed from the culture vessels, spread in a rather thin layer, and permitted to air dry at room temperature. This requires several days. After drying the material may be stored for at least several months without injury. For many purposes it is not necessary to separate the spores from the dried bran medium. However when such separation is desired several rapid methods are available.

Probably the simplest procedure is to place the dried medium in an ordinary flour sifter, place the sifter tightly in contact with the collecting paper or pan, cover the top, and turn the sifter handle. The spores are detached from the dried bran and collected below the screen. This procedure is dusty and does not give a complete recovery. The following method is much cleaner and more efficient.

Figure 1 illustrates an apparatus devised specifically for collecting the spores. The principle of operation is that compressed air under moderate pressure agitates the dried medium contained in a separatory funnel (500 ml. size), thereby detaching the spores and also suspending them in the air stream. The air escapes from the funnel mouth through a glass tube, the latter

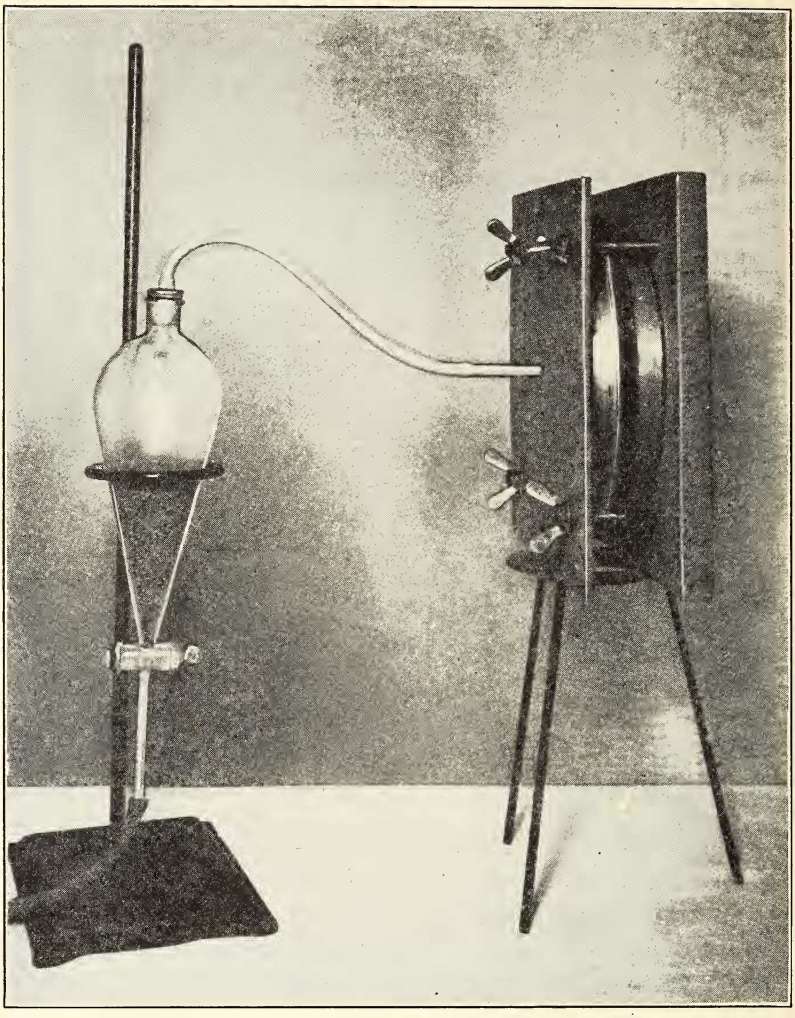

Figure 1.

being held in place by a tightly fitting rubber stopper. The glass tube extends about 4 cms. into the funnel, this end being capped by a thimble of insect screen (16 mesh per inch) to prevent the passage of bran particles. The air stream is conducted through a short rubber tube to the filter, where the spores are collected. The filter consists of two aluminum pie pans, each 23 cms. in

diameter, which, when opposed, clamp a disc of filter paper between the two rims. In order to assure a tight seal, the rim of one pan has a sponge rubber gasket cemented to the circumference. A central hole is drilled in each pan, and a short length of glass tubing cemented in each, to act respectively as inlet and escape ports for the filter. The filter paper is placed between the pans, and the assembly held tightly together by means of a simple wooden clamp.

In operation the dried medium is placed in the funnel through the mouth, the stopper replaced (thus connecting the filter) and the air pressure turned on by means of the stopcock. The pressure should be sufficient to thoroughly agitate the contents of the funnel. Within several minutes almost all of the spores are blown out of the residual bran. The residue is removed, and a fresh charge placed in the funnel. With a filter area of approximately 300 sq. cms. about 10 gms. of spores may be collected before the filter must be opened and the spores scraped from the paper. The paper may be used repeatedly. It has been found best to lightly crush the dried medium before placing it in the separator. This may be easily and cleanly done by a very short grinding in a closed ball mill.

This method of separation and collection of fungous spores is obviously not confined to any particular fungus.

SUMMARY

A procedure for culturing and obtaining large quantities of spores of the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. has been described. It is suggested that the methods are adaptable to use with a number of other fungi. The fungus is cultured on autoclaved, moistened wheat bran. The yield of spores is much heavier than occurs on commonly used culture media. The spores may be readily obtained in a practically pure condition by an air separation and filtration method. The construction of a simple separator and filter is described.

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